

Combinatorial analysis of interacting RNA molecules

Thomas J. X. Li, Christian M. Reidys*

Center for Combinatorics, LPMC-TJKLC
Nankai University
Tianjin 300071
P.R. China
Phone: *86-22-2350-6800
Fax: *86-22-2350-9272
duck@santafe.edu

Abstract

Recently several minimum free energy (MFE) folding algorithms for predicting the joint structure of two interacting RNA molecules have been proposed. Their folding targets are interaction structures, that can be represented as diagrams with two backbones drawn horizontally on top of each other such that (1) intramolecular and intermolecular bonds are noncrossing and (2) there is no “zig-zag” configuration. This paper studies joint structures with arc-length at least four in which both, interior and exterior stack-lengths are at least two (no isolated arcs). The key idea in this paper is to consider a new type of shape, based on which joint structures can be derived via symbolic enumeration. Our results imply simple asymptotic formulas for the number of joint structures with surprisingly small exponential growth rates. They are of interest in the context of designing prediction algorithms for RNA-RNA interactions.

Keywords: RNA-RNA interaction, Joint structure, Shape, Symbolic enumeration, Singularity analysis, RNA secondary structure

2010 MSC: 05A16, 92E10

1. INTRODUCTION

RNA-RNA binding is an important phenomenon observed in various classes of non-coding RNAs and plays a crucial role in a number of regulation processes. Regulatory antisense RNAs control gene expression by prohibiting the translation of a target mRNA through establishing stable base pairing interactions. Examples include the regulation of translation in both: prokaryotes [1] and eukaryotes [2, 3], the targeting of chemical modifications [4], insertion editing [5], and transcriptional control [6]. More and more evidence suggests, that RNA-RNA interactions also play a role for the functionality of long mRNA—like ncRNAs [7]. A common theme in many RNA classes, including miRNAs, snRNAs, gRNAs, snoRNAs, and in particular many of the procaryotic small RNAs, is the formation of RNA-RNA interaction structures that are much more complex than simple complementary sense-antisense interactions. The interaction between two RNAs is governed by the same physical principles that determine RNA folding: the formation of specific base pairs patterns whose energy is largely determined by base pair stacking and loop strains. As a result, secondary structures are an appropriate level of description to quantitatively understand the thermodynamics of RNA-RNA binding.

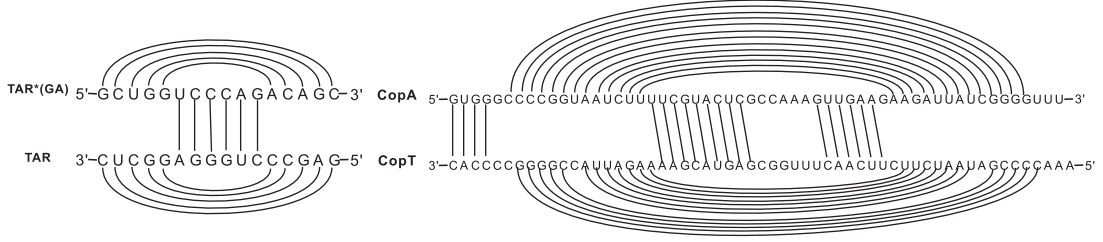


Fig. 1. Natural joint structures. Known interaction bonds of TAR*(GA)-TAR [10] and CopA-CopT [11] are displayed.

Alkan *et al.* [8] proved that the RNA-RNA interaction prediction (RIP) problem is in its general form NP-complete. Nevertheless, we are facing increasing demand for efficient computational methods for RIP. By restricting the space of allowed configurations, polynomial-time algorithms on secondary structure level have been derived. Pervouchine [9] and Alkan *et al.* [8] proposed MFE folding algorithms for predicting the *joint structure* of two interacting RNA molecules. In this model, “joint structure” means that the intramolecular structures of each partner are pseudoknot-free, that the intermolecular binding pairs are noncrossing, and that there is no so-called “zig-zag” configuration, see Fig. 1. The optimal joint structure can be computed in $O(N^6)$ time and $O(N^4)$ space by means of dynamic programming [8, 9, 12, 13]. Extensions involving the partition function were proposed by Chitsaz *et al.* (piRNA) [13] and Huang *et al.* (rip) [14]. In contrast to the situation for RNA secondary structures [15, 16], much less is known about joint structures. Only joint structures of arc-length greater than or equal to two have been studied in [17]. However, the biochemistry of nucleotide-pairings, favors parallel stacking of bonds due to entropy and the minimum length of intramolecular bonds of four. Unfortunately, the biophysically relevant class of canonical joint structures with arc-length ≥ 4 , is not governed by the framework in [17].

In this paper, we introduce the general framework dealing with σ -canonical joint structures having arc-length $\geq \sigma + 2$. In particular, our results apply to the class of canonical joint structures having arc-length ≥ 4 . Our results are relevant for the design and analysis of RIP folding algorithms and show that the numbers of σ -canonical joint structures with arc-length $\geq \sigma + 2$ exhibit surprisingly small exponential growth rates.

This paper is organized as follows: in Section 2 we introduce joint structures along the lines of [12] and in Section 3 we compute, along the lines of [18], the generating function of refined shapes via symbolic enumeration. In Section 4 we show how to inflate refined shapes into joint structures and derive the generating function of joint structures. Section 5 presents the singularity analysis and asymptotic formulas. We finally integrate our results in Section 6.

2. SECONDARY STRUCTURES AND JOINT STRUCTURES

Let us begin by discussing some basic results of [15, 16, 19]. Let $f(n)$ denote the number of all noncrossing matchings of n arcs having the generating function $\mathbf{F}(z) = \sum f(n) z^n$.

Recursions for $f(n)$ allow us to derive $z\mathbf{F}(z)^2 - \mathbf{F}(z) + 1 = 0$, that is we have

$$\mathbf{F}(z) = \frac{1 - \sqrt{1 - 4z}}{2z}.$$

Let $\mathcal{T}_\sigma^{[\lambda]}$ denote the combinatorial class of σ -canonical secondary structures having arc-length $\geq \lambda$ and $T_\sigma^{[\lambda]}(n)$ denote the number of all σ -canonical secondary structures over n vertices having arc-length $\geq \lambda$ and

$$\mathbf{T}_\sigma^{[\lambda]}(z) = \sum T_\sigma^{[\lambda]}(n) z^n.$$

Theorem 1. [19] *Let $\sigma \in \mathbb{N}$, z be an indeterminant and let*

$$\begin{aligned} u_\sigma(z) &= \frac{(z^2)^{\sigma-1}}{z^{2\sigma} - z^2 + 1}, \\ v_\lambda(z) &= 1 - z + u_\sigma(z) \sum_{h=2}^{\lambda} z^h, \end{aligned}$$

then, $\mathbf{T}_\sigma^{[\lambda]}(z)$, the generating function of σ -canonical structures with minimum arc-length λ is given by

$$\mathbf{T}_\sigma^{[\lambda]}(z) = \frac{1}{v_\lambda(z)} \mathbf{F} \left(\left(\frac{\sqrt{u_\sigma(z)} z}{v_\lambda(z)} \right)^2 \right),$$

where

$$\mathbf{F}(z) = \frac{1 - \sqrt{1 - 4z}}{2z}.$$

Furthermore

$$T_\sigma^{[\lambda]}(n) \sim c_\sigma^{[\lambda]} n^{-\frac{3}{2}} \left(\frac{1}{\zeta_\sigma^{[\lambda]}} \right)^n,$$

where $\zeta_\sigma^{[\lambda]}$ is the dominant singularity of $\mathbf{T}_\sigma^{[\lambda]}(z)$ and the minimal positive real solution of the equation

$$\left(\frac{\sqrt{u_\sigma(z)} z}{v_\lambda(z)} \right)^2 = \frac{1}{4}.$$

Theorem 1 implies that for any specified λ and σ , $\mathbf{T}_\sigma^{[\lambda]}(z)$ is algebraic over the rational function field $\mathbb{C}(z)$, since $\mathbf{F}(z)$ is algebraic and $v_\lambda(z), u_\sigma(z)$ are both rational functions.

Given two RNA sequences $R = (R_i)_1^n$ and $S = (S_j)_1^m$ with n and m vertices, we index the vertices such that R_1 is the 5' end of R and S_1 is the 3' end of S . The intramolecular base pair can be represented by an arc (interior), with its two endpoints contained in either R or S . Similarly, the extramolecular base pair can be represented by an arc (exterior) with one of its endpoints contained in R and the other in S . When representing arc-configurations, we draw all R -arcs in the upper-halfplane and all S -arcs in the lower-halfplane, see Fig. 2, **(A)**.

We refer to the subgraph induced by $\{R_i, \dots, R_j\}$ by $R[i, j]$. The subgraph $R[i, j](S[i', j'])$ is called *secondary segment* if there is no exterior arc $R_k S_{k'}$ such that $i \leq k \leq j$

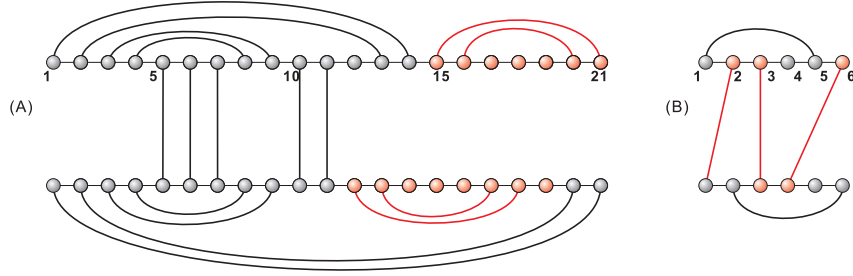


Fig. 2. (A): A joint structure $J(R, S, I)$ with arc-length ≥ 4 and stack-length ≥ 2 . Secondary segments (red): the subgraphs $R[15, 21]$ and $S[12, 19]$. Ancestors and descendants: for the exterior arc R_5S_5 , we have the following sets of R -ancestors and S -ancestors of R_5S_5 : $\{R_1R_{14}, R_2R_{13}, R_3R_9, R_4R_8\}$ and $\{S_1S_{21}, S_2S_{20}, S_3S_9, S_4S_8\}$. The exterior arc R_5S_5 is a common descendant of R_1R_{14} and S_3S_9 , while $R_{10}S_{10}$ is not. Subsumed arcs: R_1R_{14} subsumes S_3S_9 and S_1S_{21} . (B): A zigzag, generated by R_2S_1 , R_3S_3 and R_6S_4 .

($i' \leq k' \leq j'$), see Fig. 2, (A). An interior arc R_iR_j is an R -ancestor of the exterior arc $R_kS_{k'}$ if $i < k < j$. Analogously, $S_{i'}S_{j'}$ is an S -ancestor of $R_kS_{k'}$ if $i' < k' < j'$. We also refer to $R_kS_{k'}$ as a *descendant* of R_iR_j and $S_{i'}S_{j'}$ in this situation, see Fig. 2, (A). Furthermore, we call R_iR_j and $S_{i'}S_{j'}$ *dependent* if they have a common descendant and *independent*, otherwise. Let R_iR_j and $S_{i'}S_{j'}$ be two dependent interior arcs. Then R_iR_j *subsumes* $S_{i'}S_{j'}$, or $S_{i'}S_{j'}$ is subsumed in R_iR_j , if for any $R_kS_{k'} \in I$, $i' < k' < j'$ implies $i < k < j$, that is, the set of descendants of $S_{i'}S_{j'}$ is contained in the set of descendants of R_iR_j , see Fig. 2, (A). A *zigzag* is a subgraph containing two dependent interior arcs R_iR_j and $S_{i'}S_{j'}$ neither one subsuming the other, see Fig. 2, (B).

A *joint structure* [9, 8, 13, 12] $J(R, S, I)$, see Fig. 2, (A), is a graph such that

- (1) R, S are secondary structures (each nucleotide being paired with at most one other nucleotide via hydrogen bonds, without internal pseudoknots);
- (2) I is a set of exterior arcs without external pseudoknots, i.e., if $R_iS_j, R_{i'}S_{j'} \in I$ then $i < i'$ implies $j < j'$;
- (3) $J(R, S, I)$ contains no zig-zags.

We next specify some notations

- an interior arc (or simply arc) of length λ is an arc R_iR_j ($S_{i'}S_{j'}$) where $j - i = \lambda$ ($j' - i' = \lambda$),
- an interior stack (or simply stack) of length σ is a maximal sequence of “parallel” interior arcs,

$$(R_iR_j, R_{i+1}R_{j-1}, \dots, R_{i+\sigma-1}R_{j-\sigma+1}) \quad \text{or} \\ (S_iS_j, S_{i+1}S_{j-1}, \dots, S_{i+\sigma-1}S_{j-\sigma+1}),$$

- an exterior stack of length τ is a maximal sequence of “parallel” exterior arcs,

$$(R_iS_{i'}, R_{i+1}S_{i'+1}, \dots, R_{i+\tau-1}S_{i'+\tau-1}).$$

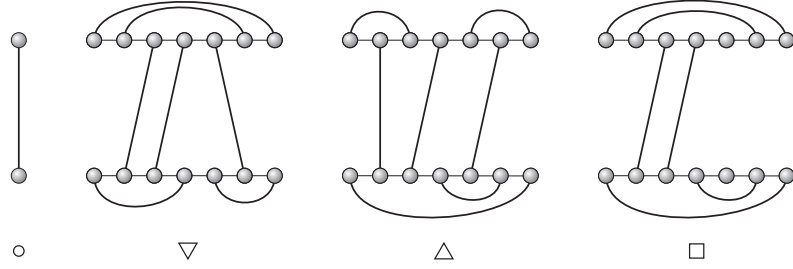


Fig. 3. The four types of tight structures are defined as follows: $\circ : \{R_i S_{i'}\} = J_{i,j;i',j'}^\circ$ and $i = j, i' = j'$; $\nabla : R_i R_j \in J_{i,j;i',j'}^\nabla$ and $S_{i'} S_{j'} \notin J_{i,j;i',j'}^\nabla$; $\Delta : S_{i'} S_{j'} \in J_{i,j;i',j'}^\Delta$ and $R_i R_j \notin J_{i,j;i',j'}^\Delta$; $\square : \{R_i R_j, S_{i'} S_{j'}\} \in J_{i,j;i',j'}^\square$;

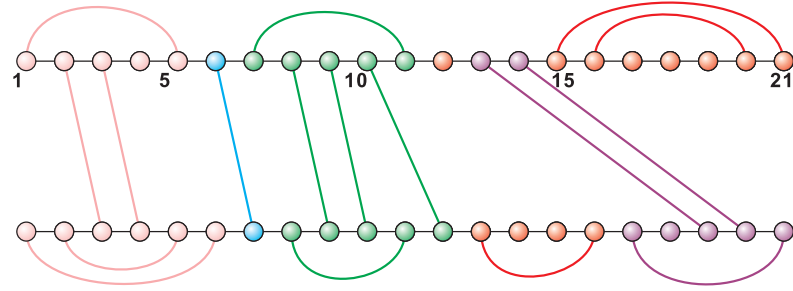


Fig. 4. Decomposition of joint structures. We display different secondary segments (red) and tight structures (\circ blue, ∇ green, Δ purple, \square pink) in which $J_{1,21;1,21}$ decomposes.

A σ -canonical joint structure is a joint structure with stack-length $\geq \sigma$. In Fig. 2, (A), we give an example of 2-canonical joint structure with arc-length ≥ 4 .

Let the block $J_{i,j;i',j'}$ denote the subgraph of a joint structure $J(R, S, I)$ induced by a pair of subsequences $\{R_i, R_{i+1}, \dots, R_j\}$ and $\{S_{i'}, S_{i'+1}, \dots, S_{j'}\}$. Given a joint structure $J(R, S, I)$, a *tight* structure of $J(R, S, I)$ is the minimal block $J_{i,j;i',j'}$ containing all the R -ancestors and S -ancestors of any exterior arc in $J_{i,j;i',j'}$ and all the descendants of any interior arc in $J_{i,j;i',j'}$. In the following, a tight structure is denoted by $J_{i,j;i',j'}^T$. In particular, we denote the joint structure $J(R, S, I)$ by $J^T(R, S, I)$ if $J(R, S, I)$ is a tight structure of itself. For any joint structure, there are only four types of tight structures $J_{i,j;i',j'}^T$, that is $\{\circ, \nabla, \Delta, \square\}$, denoted by $J_{i,j;i',j'}^{\{\circ, \nabla, \Delta, \square\}}$, respectively, see Fig. 3.

The key function of tight structures is that they are the building blocks for the decomposition of joint structures.

Proposition 1. [12] *Let $J(R, S, I)$ be a joint structure. Then*

- (1) *any exterior arc $R_k S_{k'}$ in $J(R, S, I)$ is contained in a unique tight structure.*
- (2) *$J(R, S, I)$ decomposes into a unique collection of tight structures and maximal secondary segments.*

In Fig. 4 we illustrate the decomposition of a joint structure.

3. REFINED SHAPES

A *shape* [17] is a joint structure containing no secondary segments in which each interior stack and each exterior stack have length exactly one. We follow the ideas in [18] and obtain the generating function of joint structures via inflation of refined (colored) shapes. Refined shapes are obtained by distinguishing two classes of exterior shape-arcs. Each distinguished class requires its specific inflation-procedure (see Theorem 2). Let us have a closer look at two particular classes of exterior arcs:

- **Class \mathbf{A}_1 :** the class of arc-pairs (α, β) where α is an exterior arc with the unique interior 2-arc β as its ancestor.
- **Class \mathbf{A}_2 :** the class of arc-triples (α, β, γ) where α is an exterior arc with interior 2-arcs β and γ as ancestors.

Let \mathcal{G} denote the combinatorial class of shapes. Given a joint structure, we can obtain its shape by first removing all secondary segments and second collapsing any stack into a single arc. That is, we have a map $\varphi: \mathcal{J} \rightarrow \mathcal{G}$. In Fig. 5, we illustrate how a joint structure is projected into its refined shape. The resulting shape exhibits elements in class \mathbf{A}_1 as well as class \mathbf{A}_2 .

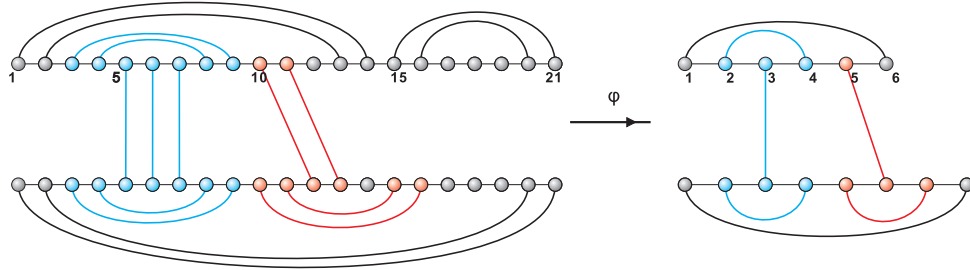


Fig. 5. Joint structures and their refined shapes: a 2-canonical joint structure with arc-length ≥ 4 (left) is projected to its refined shape (right), which exhibits elements of class \mathbf{A}_1 (red) and \mathbf{A}_2 (blue), respectively.

Let $G(t, h, a_1, a_2)$ denote the number of shapes having total t interior arcs and h exterior arcs containing a_1 elements of class \mathbf{A}_1 and a_2 elements of class \mathbf{A}_2 and

$$\mathbf{G}(x, z, u, v) = \sum_{t, h, a_1, a_2} G(t, h, a_1, a_2) x^t z^h u^{a_1} v^{a_2}.$$

We shall proceed by revisiting the notions of tight shapes, double tight shapes, interaction segments, closed shapes and right closed shapes [12]:

- a tight shape is tight as a structure. Let \mathcal{G}^T denote the class of tight shapes and $G^T(t, h, a_1, a_2)$ denote the number of tight shapes having total t interior arcs and h exterior arcs containing a_1 elements of class \mathbf{A}_1 and a_2 elements of class \mathbf{A}_2 ,

$$\mathbf{G}^T(x, z, u, v) = \sum G^T(t, h, a_1, a_2) x^t z^h u^{a_1} v^{a_2}.$$

Any tight shape, comes as exactly one of the four types $\{\circ, \nabla, \Delta, \square\}$. The corresponding classes and generating functions are defined accordingly, $\mathcal{G}^{\{\circ, \nabla, \Delta, \square\}}$ and $\mathbf{G}^{\{\circ, \nabla, \Delta, \square\}}(x, z, u, v)$ respectively,

- a double tight shape is a shape whose leftmost and rightmost blocks are tight structures. Let \mathcal{G}^{DT} denote the class of double tight shapes and $G^{DT}(t, h, a_1, a_2)$ denote the number of double tight shapes having total t interior arcs and h exterior arcs containing a_1 elements of class \mathbf{A}_1 and a_2 elements of class \mathbf{A}_2 ,

$$\mathbf{G}^{DT}(x, z, u, v) = \sum G^{DT}(t, h, a_1, a_2) x^t z^h u^{a_1} v^{a_2},$$

- a closed shape is a tight shape of type $\{\nabla, \Delta, \square\}$. Let \mathcal{G}^C denote the class of closed shapes and $G^C(t, h, a_1, a_2)$ denote the number of closed shapes having total t interior arcs and h exterior arcs containing a_1 elements of class \mathbf{A}_1 and a_2 elements of class \mathbf{A}_2 ,

$$\mathbf{G}^C(x, z, u, v) = \sum G^C(t, h, a_1, a_2) x^t z^h u^{a_1} v^{a_2},$$

- a right closed shape is a shape whose rightmost block is a closed shape rather than an exterior arc. Let \mathcal{G}^{RC} denote the class of right closed shapes and $G^{RC}(t, h, a_1, a_2)$ denote the number of right close shapes having total t interior arcs and h exterior arcs containing a_1 elements of class \mathbf{A}_1 and a_2 elements of class \mathbf{A}_2 ,

$$\mathbf{G}^{RC}(x, z, u, v) = \sum G^{RC}(t, h, a_1, a_2) x^t z^h u^{a_1} v^{a_2},$$

- in a shape, an interaction segment is an empty structure or a tight structure of type \circ (an exterior arc). We denote the class of interaction segment by \mathcal{I} and the associated generating function by $\mathbf{I}(x, z, u, v)$. Obviously, $\mathbf{I}(x, z, u, v) = 1 + z$.

Lemma 1. *The generating function $\mathbf{G}(x, z, u, v)$ of refined shapes satisfies*

$$(3.1) \quad \mathbf{A}(x, z) \mathbf{G}(x, z, u, v)^2 + \mathbf{B}(x, z, u, v) \mathbf{G}(x, z, u, v) + \mathbf{C}(x, z) = 0,$$

where

$$(3.2) \quad \begin{aligned} \mathbf{A}(x, z) &= x(x+2)(z+1), \\ \mathbf{B}(x, z, u, v) &= -\left(x(x+2)(z+1)^2 + (x+1)^2 - (2xu + x^2v)z(z+1)\right), \\ \mathbf{C}(x, z) &= (x+1)^2(z+1). \end{aligned}$$

Explicitly,

$$(3.3) \quad \mathbf{G}(x, z, u, v) = \frac{-\mathbf{B}(x, z, u, v) - \sqrt{\mathbf{B}(x, z, u, v)^2 - 4\mathbf{A}(x, z)\mathbf{C}(x, z)}}{2\mathbf{A}(x, z)}.$$

Proof. Proposition 1 implies that any shape can be decomposed into a unique collection of tight shapes. Furthermore, each shape can be decomposed into a unique collection of closed shapes and exterior arcs. We decompose a shape in four steps, see Fig. 6. We translate each decomposition step into the construction of combinatorial classes.

Step (1): decomposition into a right closed shape and its rightmost interaction segment, i.e.

$$\mathcal{G} = \mathcal{G}^{RC} \times \mathcal{I} + \mathcal{I}.$$

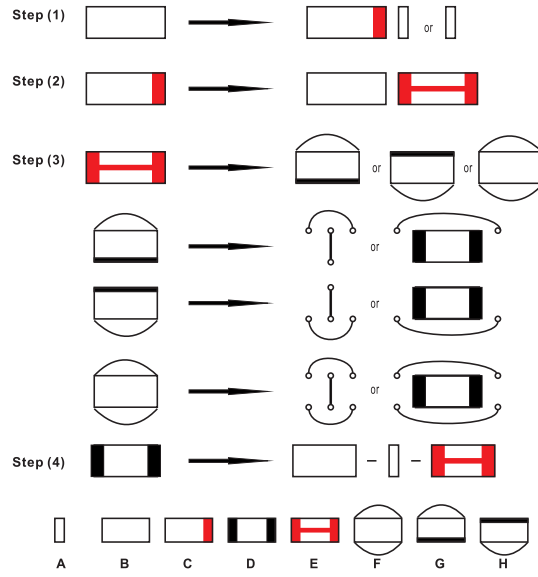


Fig. 6. The shape-grammar. The notations of structural components are explained in the panel below. **A**: interaction segment; **B**: arbitrary shape $G(R, S, I)$; **C**: right closed shape $G^{RC}(R, S, I)$; **D**: double tight shape $G^{DT}(R, S, I)$; **E**: closed shape $G^C(R, S, I)$; **F**: type \square tight shape $G^\square(R, S, I)$; **G**: type ∇ tight shape $G^\nabla(R, S, I)$; **H**: type Δ tight shape $G^\Delta(R, S, I)$; **I**: type \circ tight shape $G^\circ(R, S, I)$;

and Proposition 2 implies

$$(3.4) \quad \mathbf{G}(x, z, u, v) = \mathbf{G}^{RC}(x, z, u, v) \cdot \mathbf{I}(x, z, u, v) + \mathbf{I}(x, z, u, v).$$

Step (2): splitting of the rightmost closed shape in a right closed shape

$$\mathcal{G}^{RC} = \mathcal{G} \times \mathcal{G}^C,$$

whence

$$(3.5) \quad \mathbf{G}^{RC}(x, z, u, v) = \mathbf{G}(x, z, u, v) \cdot \mathbf{G}^C(x, z, u, v).$$

Step (3): type-dependent decomposition of a closed shape.

$$\begin{aligned} \mathcal{G}^C &= \mathcal{G}^\nabla + \mathcal{G}^\Delta + \mathcal{G}^\square \\ \mathcal{G}^\nabla &= (\mathcal{Z}, \mathcal{Z}, \mathcal{Z}, \mathcal{E}) + (\mathcal{Z}, \mathcal{E}, \mathcal{E}, \mathcal{E}) \times \mathcal{G}^{DT} \\ \mathcal{G}^\Delta &= (\mathcal{Z}, \mathcal{Z}, \mathcal{Z}, \mathcal{E}) + (\mathcal{Z}, \mathcal{E}, \mathcal{E}, \mathcal{E}) \times \mathcal{G}^{DT} \\ \mathcal{G}^\square &= (\mathcal{Z} \times \mathcal{Z}, \mathcal{Z}, \mathcal{E}, \mathcal{Z}) + (\mathcal{Z} \times \mathcal{Z}, \mathcal{E}, \mathcal{E}) \times \mathcal{G}^{DT}. \end{aligned}$$

We therefore have

$$\begin{aligned} \mathbf{G}^C(x, z, u, v) &= \mathbf{G}^\nabla(x, z, u, v) + \mathbf{G}^\Delta(x, z, u, v) + \mathbf{G}^\square(x, z, u, v) \\ \mathbf{G}^\nabla(x, z, u, v) &= xzu + x\mathbf{G}^{DT}(x, z, u, v) \\ \mathbf{G}^\Delta(x, z, u, v) &= xzu + x\mathbf{G}^{DT}(x, z, u, v) \\ \mathbf{G}^\square(x, z, u, v) &= x^2zv + x^2\mathbf{G}^{DT}(x, z, u, v). \end{aligned} \quad (3.6)$$

Step (4): obtaining double tight shapes of Step (3) by excluding the class of interaction segment and the class of closed shapes, i.e.

$$\mathcal{G}^{DT} = \mathcal{G} - \mathcal{I} - \mathcal{G}^C,$$

whence

$$(3.7) \quad \mathbf{G}^{DT}(x, z, u, v) = \mathbf{G}(x, z, u, v) - \mathbf{I}(x, z, u, v) - \mathbf{G}^C(x, z, u, v).$$

Solving equations (3.4)–(3.7), leads to (1) the functional equation (3.1) and (2) eq. (3.2). This quadratic equation together with the initial condition $\mathbf{G}(0, 0, 0, 0) = 1$, implies eq. (3.3). \square

4. THE GENERATING FUNCTION OF JOINT STRUCTURES

Let \mathcal{H}_σ denote the class of σ -canonical joint structures with arc-length $\geq \sigma + 2$. Let $H_\sigma(s)$ denote the number of joint structures in \mathcal{H}_σ with total s vertices having the generating function

$$\mathbf{H}_\sigma(x) = \sum H_\sigma(s) x^s.$$

We are now in position to compute the generating function $\mathbf{H}_\sigma(x)$. Our strategy is inflating the shapes with specific inflation on specific exterior arcs.

Theorem 2. *For any $\sigma \geq 1$, $\mathbf{H}_\sigma(x)$ is a power series and*

$$(4.1) \quad \mathbf{H}_\sigma(x) = \mathbf{T}_\sigma^{[\sigma+2]}(x)^2 \mathbf{G}(\eta, \eta, \eta_1, \eta_2),$$

where

$$\begin{aligned} \eta &= \frac{x^{2\sigma} \mathbf{T}_\sigma^{[\sigma+2]}(x)^2}{1 - x^2 - x^{2\sigma}(\mathbf{T}_\sigma^{[\sigma+2]}(x)^2 - 1)}, \\ \eta_1 &= \frac{-1 + x^2 - x^{2\sigma} + (1 + x^{2\sigma})\mathbf{T}_\sigma^{[\sigma+2]}(x)^2}{\mathbf{T}_\sigma^{[\sigma+2]}(x)^2}, \\ \eta_2 &= \frac{1 - x^2 + x^{2\sigma} + (-2 + 2x^2 - 3x^{2\sigma})\mathbf{T}_\sigma^{[\sigma+2]}(x)^2 + (1 + 2x^{2\sigma})\mathbf{T}_\sigma^{[\sigma+2]}(x)^4}{\mathbf{T}_\sigma^{[\sigma+2]}(x)^4}. \end{aligned}$$

Proof. Let $\mathcal{G}(t, h, a_1, a_2)$ denote the class of shapes having total t interior arcs and h exterior arcs containing a_1 elements of class \mathbf{A}_1 and a_2 elements of class \mathbf{A}_2 . For any joint structure in \mathcal{H}_σ , we obtain a shape in \mathcal{G} as follows:

- (1) remove all secondary segments,
- (2) collapse each interior stack into one interior arc and each exterior stack into one exterior arc.

Then we have the surjective map

$$\varphi: \mathcal{H}_\sigma \rightarrow \mathcal{G}.$$

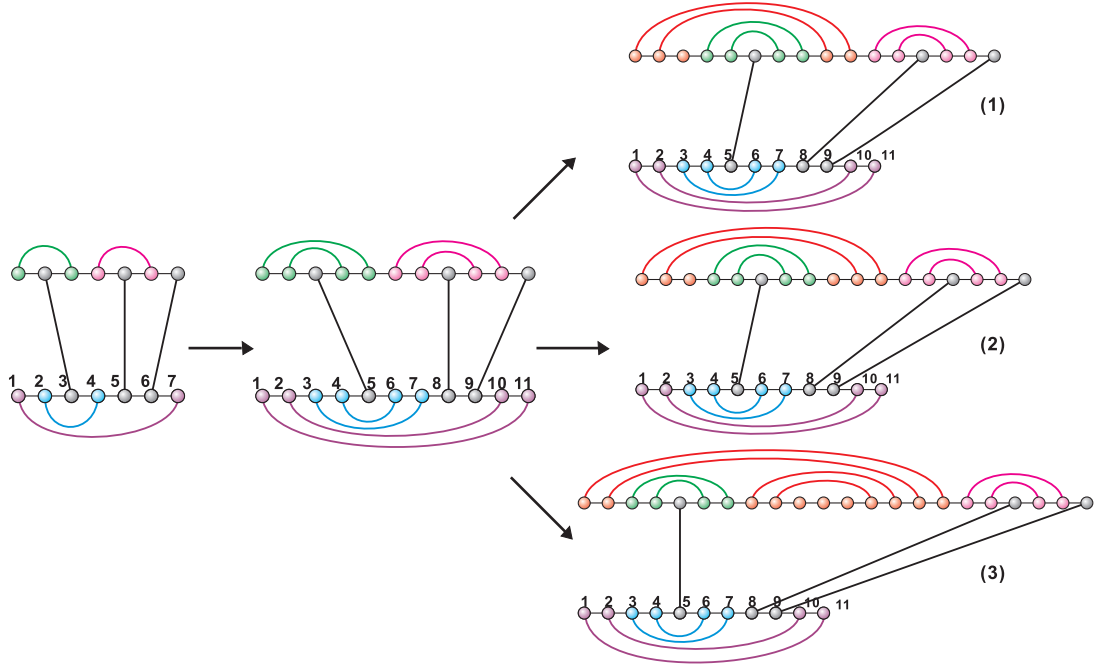


Fig. 7. Step I: a shape (left) is inflated to a joint structure with arc-length ≥ 2 and interior stack-length ≥ 2 . Each interior arc in the shape is first inflated to a stack of size at least two (middle) and second inflated by adding one induced stack of size two (right). Note that there are three ways to insert the secondary segments to separate the induced stacks (red).

Indeed, for any shape γ in \mathcal{G} , we can construct σ -canonical joint structures with arc-length $\geq \sigma + 2$. $\varphi: \mathcal{H}_\sigma \rightarrow \mathcal{G}$, induces the partition $\mathcal{H}_\sigma = \dot{\cup}_\gamma \varphi^{-1}(\gamma)$, whence

$$(4.2) \quad \mathbf{H}_\sigma(x) = \sum_{\gamma \in \mathcal{G}} \mathbf{H}_\gamma(x),$$

where $\mathbf{H}_\gamma(x)$ denotes the generating function of joint structures having shape γ . We proceed by computing the generating function $\mathbf{H}_\gamma(x)$. We will construct $\mathbf{H}_\gamma(x)$ via simpler combinatorial classes as building blocks considering stems, stacks, induced stacks, interior arcs, exterior arcs and secondary segments. We inflate a shape γ in $\mathcal{G}(t, h, a_1, a_2)$ to a joint structure in \mathcal{H}_σ in four steps.

Step I: we inflate any interior arc in γ to a stack of size at least σ and subsequently add additional stacks. The latter are called induced stacks and have to be separated by means of inserting secondary segments, see Fig. 7. Note that during this first inflation step no secondary segments, other than those necessary for separating the nested stacks are inserted. We generate

- secondary segments $\mathcal{T}_\sigma^{[\sigma+2]}$ having arc-length $\geq \sigma + 2$ and stack-length $\geq \sigma$ with generating function $\mathbf{T}_\sigma^{[\sigma+2]}(x)$,
- interior arcs \mathcal{R} with generating function $\mathbf{R}(x) = x^2$,

- stacks, i.e. pairs consisting of the minimal sequence of arcs \mathcal{R}^σ and an arbitrary extension consisting of arcs of arbitrary finite length

$$\mathcal{K}_\sigma = \mathcal{R}^\sigma \times \text{SEQ}(\mathcal{R}),$$

having the generating function

$$\mathbf{K}_\sigma(x) = x^{2\sigma} \cdot \frac{1}{1-x^2},$$

- induced stacks, i.e. stacks together with at least one secondary segment on either or both of its sides,

$$\mathcal{N}_\sigma = \mathcal{K}_\sigma \times ((\mathcal{T}_\sigma^{[\sigma+2]})^2 - 1),$$

having the generating function

$$\mathbf{N}_\sigma(x) = \frac{x^{2\sigma}}{1-x^2} (\mathbf{T}_\sigma^{[\sigma+2]}(x)^2 - 1),$$

- stems, that is pairs consisting of stacks \mathcal{K}_σ and an arbitrarily long sequence of induced stacks

$$\mathcal{M}_\sigma = \mathcal{K}_\sigma \times \text{SEQ}(\mathcal{N}_\sigma),$$

having the generating function

$$\mathbf{M}_\sigma(x) = \frac{\mathbf{K}_\sigma(x)}{1 - \mathbf{N}_\sigma(x)} = \frac{\frac{x^{2\sigma}}{1-x^2}}{1 - \frac{x^{2\sigma}}{1-x^2} (\mathbf{T}_\sigma^{[\sigma+2]}(x)^2 - 1)}.$$

Note that we inflate both, top and bottom sequences. The corresponding generating function is $\mathbf{M}_\sigma(x)^t$.

Step II: we inflate any exterior arc in γ , but not as an element of classes \mathbf{A}_1 or \mathbf{A}_2 , to an exterior stack of size at least σ and subsequently add additional exterior stacks. The latter are called induced exterior stacks and have to be separated by means of inserting secondary segments, see Fig. 8. Note that during this exterior-arc inflation no secondary segments, other than those necessary for separating the stacks are inserted. We generate

- exterior arc \mathcal{R}_0 having the generating function $\mathbf{R}_0 = x^2$,
- exterior stacks, i.e. pairs consisting of the minimal sequence of exterior arcs \mathcal{R}_0^σ and an arbitrary extension consisting of exterior arcs of arbitrary finite length

$$\mathcal{K}_\sigma^* = \mathcal{R}_0^\sigma \times \text{SEQ}(\mathcal{R}_0),$$

having the generating function

$$\mathbf{K}_\sigma^*(x) = x^{2\sigma} \cdot \frac{1}{1-x^2},$$

- induced exterior stacks, i.e. stacks together with at least one secondary segment on either or both its sides,

$$\mathcal{N}_\sigma^* = \mathcal{K}_\sigma^* \times ((\mathcal{T}_\sigma^{[\sigma+2]})^2 - 1),$$

having the generating function

$$\mathbf{N}_\sigma^*(x) = \frac{x^{2\sigma}}{1-x^2} (\mathbf{T}_\sigma^{[\sigma+2]}(x)^2 - 1),$$

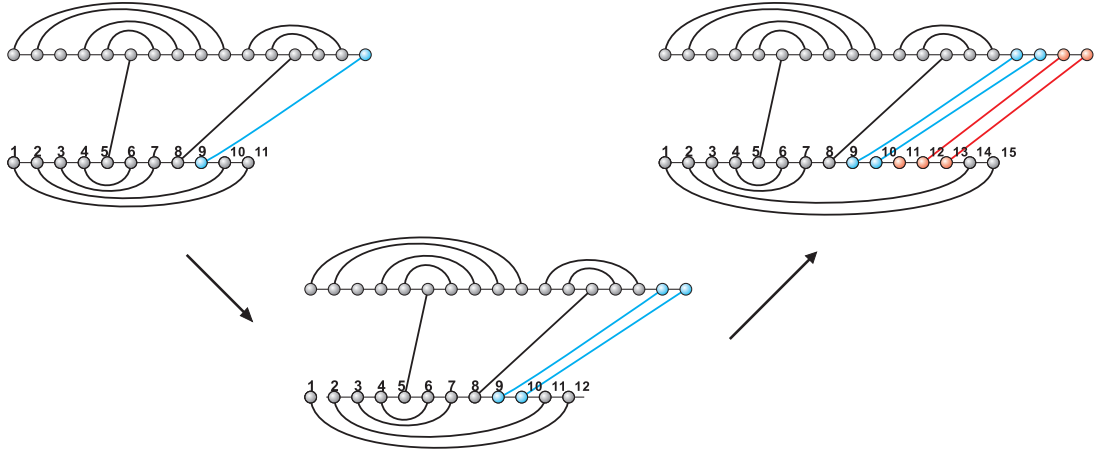


Fig. 8. Step II: a joint structure (top-left) obtained in (1) in Fig. 7 is inflated to a new joint structure. Each exterior arc, not contained in classes \mathbf{A}_1 or \mathbf{A}_2 , is first inflated to an exterior stack of size at least two (bottom) and second inflated by adding one exterior induced stack of size two (top-right). Note that just one of the three possible ways of inserting the secondary segments in order to separate the induced exterior stacks (red) is displayed.

- exterior stems, that is pairs consisting of exterior stacks \mathcal{K}_σ^* and an arbitrarily long sequence of induced exterior stacks

$$\mathcal{M}_\sigma^* = \mathcal{K}_\sigma^* \times \text{SEQ}(\mathcal{N}_\sigma^*),$$

having the generating function

$$\mathbf{M}_\sigma^*(x) = \frac{\mathbf{K}_\sigma^*(x)}{1 - \mathbf{N}_\sigma^*(x)} = \frac{\frac{x^{2\sigma}}{1-x^2}}{1 - \frac{x^{2\sigma}}{1-x^2} (\mathbf{T}_\sigma^{[\sigma+2]}(x)^2 - 1)}.$$

Inflating all exterior arcs that are not contained in classes \mathbf{A}_1 or \mathbf{A}_2 , we obtain $(\mathbf{M}_\sigma^*(x))^{h-a_1-a_2}$.

Step III: We inflate exterior arcs contained in classes \mathbf{A}_1 and \mathbf{A}_2 by inserting additional secondary segments at positions between the exterior arc and interior 2-arc, see Fig. 9. In contrast to Step II, specific “unwanted” scenarios are excluded. We generate

- **Class \mathbf{A}_1 :** Excluding the case where the exterior arc is inflated to an exterior stack of length σ and no additional secondary segment is inserted at the position between the exterior arc and interior 2-arc, see Fig. 10, we arrive at

$$\mathcal{M}_\sigma^* \times (\mathcal{T}_\sigma^{[\sigma+2]})^2 - \mathcal{R}_0^\sigma,$$

having the generating function

$$\mathbf{M}_\sigma^*(x) \mathbf{T}_\sigma^{[\sigma+2]}(x)^2 - x^{2\sigma}.$$

- **Class \mathbf{A}_2 :** There are three scenarios which create an interior arc of arc-length $< \sigma + 2$, see Fig. 11:

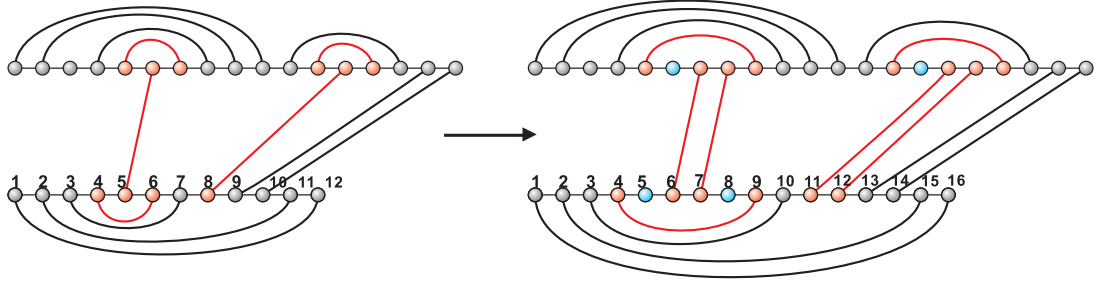


Fig. 9. Step III: a joint structure (left) obtained in the bottom in Fig. 8 is inflated to a new joint structure with arc-length ≥ 4 (right). Each exterior arc in classes \mathbf{A}_1 or \mathbf{A}_2 is inflated to an exterior stack of size at least two (red) and additional secondary segments (blue) are inserted at the positions between the exterior arc and interior 2-arc.

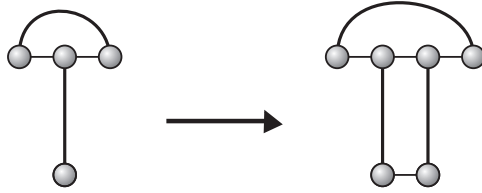


Fig. 10. “Bad” scenario for class \mathbf{A}_1 : In an element of class \mathbf{A}_1 (left), the exterior arc is inflated to an exterior stack of length 2 and no additional secondary segment is inserted at the position between the exterior arc and interior 2-arc, leading to an interior arc having arc-length < 4 (right).

- the exterior arc is inflated to an exterior stack of length σ and no additional secondary segment is inserted at the positions in both top and bottom sequences, resulting in both interior arcs having arc-length $< \sigma + 2$,
- the exterior arc is inflated to an exterior stack of length σ and additional secondary segment is inserted at the positions only in the bottom sequence, resulting in an interior arc in the top having arc-length $< \sigma + 2$,
- the exterior arc is inflated to an exterior stack of length σ and additional secondary segment is inserted at the positions only in the top sequence, resulting in an interior arc in the bottom having arc-length $< \sigma + 2$.

Excluding these three scenarios, we obtain

$$\mathcal{M}_\sigma^* \times (\mathcal{T}_\sigma^{[\sigma+2]})^4 - 2\mathcal{R}_0^\sigma \times ((\mathcal{T}_\sigma^{[\sigma+2]})^2 - \mathcal{E}) - \mathcal{R}_0^\sigma,$$

having the generating function

$$\begin{aligned} & \mathbf{M}_\sigma^*(x) \mathbf{T}_\sigma^{[\sigma+2]}(x)^4 - x^{2\sigma} (\mathbf{T}_\sigma^{[\sigma+2]}(x)^2 - 1) - x^{2\sigma} (\mathbf{T}_\sigma^{[\sigma+2]}(x)^2 - 1) - x^{2\sigma} \\ &= \mathbf{M}_\sigma^*(x) \mathbf{T}_\sigma^{[\sigma+2]}(x)^4 - x^{2\sigma} (2\mathbf{T}_\sigma^{[\sigma+2]}(x)^2 - 1). \end{aligned}$$

Applying Step III for each \mathbf{A}_1 - and \mathbf{A}_2 -element, we derive

$$(\mathbf{M}_\sigma^*(x) \mathbf{T}_\sigma^{[\sigma+2]}(x)^2 - x^{2\sigma})^{a_1} (\mathbf{M}_\sigma^*(x) \mathbf{T}_\sigma^{[\sigma+2]}(x)^4 - x^{2\sigma} (2\mathbf{T}_\sigma^{[\sigma+2]}(x)^2 - 1))^{a_2}.$$

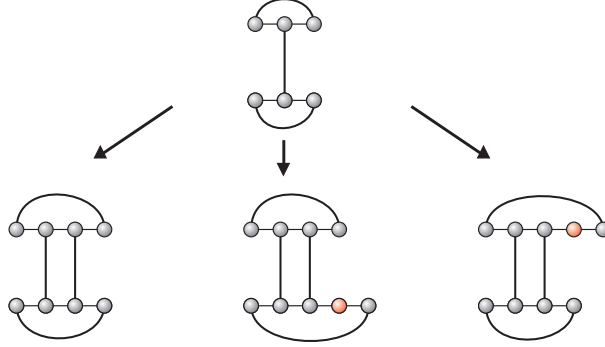


Fig. 11. “Bad” scenarios for class A_2 : In an element of class A_2 (top), there are three scenarios leading to interior arcs in both top and bottom having arc-length < 4 (bottom-left), the interior arc only in the top having arc-length < 4 (bottom-middle), the interior arc only in the bottom having arc-length < 4 (bottom-right).

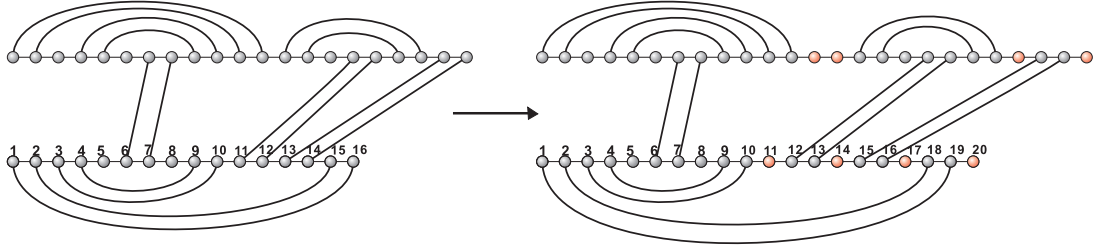


Fig. 12. Step IV: a joint structure (left) obtained in Fig. 9 is inflated to a new joint structure in \mathcal{H}_2 (right) by inserting secondary segments (red).

Step IV: Here we insert additional secondary segments at the remaining $(2t + 2h + 2 - 2a_1 - 4a_2)$ positions, see Fig. 12. Formally, this fourth inflation is expressed via the combinatorial class

$$(\mathcal{T}_\sigma^{[\sigma+2]})^{2t+2h+2-2a_1-4a_2},$$

with the generating function $(\mathbf{T}_\sigma^{[\sigma+2]}(x))^{2t+2h+2-2a_1-4a_2}$.

Combining Steps I – IV, we arrive at

$$\begin{aligned} \mathbf{H}_\gamma(x) &= \mathbf{M}_\sigma(x)^t (\mathbf{M}_\sigma^*(x))^{h-a_1-a_2} (\mathbf{M}_\sigma^*(x) \mathbf{T}_\sigma^{[\sigma+2]}(x)^2 - x^{2\sigma})^{a_1} \\ &\quad \times (\mathbf{M}_\sigma^*(x) \mathbf{T}_\sigma^{[\sigma+2]}(x)^4 - x^{2\sigma} (2\mathbf{T}_\sigma^{[\sigma+2]}(x)^2 - 1))^{a_2} (\mathbf{T}_\sigma^{[\sigma+2]}(x))^{2t+2h+2-2a_1-4a_2} \\ &= (\mathbf{T}_\sigma^{[\sigma+2]}(x))^2 (\eta)^t (\eta)^h (\eta_1)^{a_1} (\eta_2)^{a_2}, \end{aligned}$$

where

$$\begin{aligned}
\eta &= \frac{x^{2\sigma} \mathbf{T}_\sigma^{[\sigma+2]}(x)^2}{1 - x^2 - x^{2\sigma}(\mathbf{T}_\sigma^{[\sigma+2]}(x)^2 - 1)}, \\
\eta_1 &= \frac{\eta - x^{2\sigma}}{\eta} \\
&= \frac{-1 + x^2 - x^{2\sigma} + (1 + x^{2\sigma})\mathbf{T}_\sigma^{[\sigma+2]}(x)^2}{\mathbf{T}_\sigma^{[\sigma+2]}(x)^2}, \\
\eta_2 &= \frac{\eta \mathbf{T}_\sigma^{[\sigma+2]}(x)^2 - x^{2\sigma}(2\mathbf{T}_\sigma^{[\sigma+2]}(x)^2 - 1)}{\eta \mathbf{T}_\sigma^{[\sigma+2]}(x)^2} \\
&= \frac{1 - x^2 + x^{2\sigma} + (-2 + 2x^2 - 3x^{2\sigma})\mathbf{T}_\sigma^{[\sigma+2]}(x)^2 + (1 + 2x^{2\sigma})\mathbf{T}_\sigma^{[\sigma+2]}(x)^4}{\mathbf{T}_\sigma^{[\sigma+2]}(x)^4}.
\end{aligned}$$

In view of $\mathbf{T}_\sigma^{[\sigma+2]}(0) \neq 0$ the inverse $[\mathbf{T}_\sigma^{[\sigma+2]}(x)]^{-1}$ exists and accordingly η , η_1 and η_2 are welldefined. Since for any $\gamma, \gamma_1 \in \mathcal{G}(t, h, a_1, a_2)$ we have $\mathbf{H}_\gamma(x) = \mathbf{H}_{\gamma_1}(x)$, we derive

$$\mathbf{H}_\sigma(x) = \sum_{\gamma \in \mathcal{G}} \mathbf{H}_\gamma(x) = \sum_{\substack{(t, h, a_1, a_2) \\ \gamma \in \mathcal{G}(t, h, a_1, a_2)}} G(t, h, a_1, a_2) \mathbf{H}_\gamma(x).$$

Using $\mathbf{G}(x, z, u, v) = \sum G(t, h, a_1, a_2) x^t z^h u^{a_1} v^{a_2}$, we arrive at

$$\mathbf{H}_\sigma(x) = \mathbf{T}_\sigma^{[\sigma+2]}(x)^2 \mathbf{G}(\eta, \eta, \eta_1, \eta_2).$$

It remains to verify that $H_\sigma(x)$ is indeed a power series, which follows from the fact that the constant coefficients of η , η_1 and η_2 , regarded as formal power series, are zero. \square

5. ASYMPTOTIC ENUMERATION

In this section, we derive simple formulas for the number of joint structures in the limit of long sequences.

Theorem 3. *For $\sigma \geq 1$, $\mathbf{H}_\sigma(x)$ is algebraic and we have*

$$(5.1) \quad H_\sigma(s) \sim c_\sigma s^{-\frac{3}{2}} (\kappa_\sigma^{-1})^s, \quad \text{for some } c_\sigma,$$

where κ_σ is the minimal, positive real solution of the equation $\mathbf{Q}(x, \mathbf{T}_\sigma^{[\sigma+2]}(x)) = 0$, see Table 1, where

$$\begin{aligned}
\mathbf{Q}(x, y) &= (1 - x^2 + x^{2\sigma})^4 + 2x^{2\sigma} (1 - x^2 + x^{2\sigma})^2 (-3 + 3x^2 - x^{2\sigma} + x^{4\sigma} - 2x^{2+2\sigma}) y^2 \\
&\quad x^{4\sigma} (3 - 6x^2 + 3x^4 + 10x^{2\sigma} + 13x^{4\sigma} + 6x^{6\sigma} + x^{8\sigma} \\
&\quad - 14x^{2+2\sigma} + 4x^{4+2\sigma} - 14x^{2+4\sigma} + 4x^{4+4\sigma} - 4x^{2+6\sigma}) y^4 \\
&\quad - 2x^{6\sigma} (1 - x^2 + 3x^{2\sigma} + x^{4\sigma} - 2x^{2+2\sigma}) y^6 + x^{8\sigma} y^8.
\end{aligned}$$

In particular, we have $c_1 \approx 1.38629$ and $c_2 \approx 3.51610$.

σ	1	2	3	4	5
κ_σ^{-1}	3.30027	2.18096	1.82912	1.65183	1.54322

TABLE 1. Exponential growth rates κ_σ^{-1} for σ -canonical joint structures with arc-length $\geq \sigma + 2$.

Proof. Set $\mathbf{G}_1(x) = \mathbf{G}(\eta, \eta, \eta_1, \eta_2)$ and $L = \mathbb{C}(x)[\mathbf{T}_\sigma^{[\sigma+2]}(x)]$. Combining eq. (3.1) in Lemma 1 and eq. (4.1) in Theorem 2, we compute

$$\begin{aligned} \mathbf{H}_\sigma(x) &= \mathbf{T}_\sigma^{[\sigma+2]}(x)^2 \mathbf{G}(\eta, \eta, \eta_1, \eta_2) \\ &= \mathbf{T}_\sigma^{[\sigma+2]}(x)^2 \mathbf{G}_1(x), \end{aligned}$$

where $\mathbf{G}_1(x)$ satisfies the quadratic equation

$$(5.2) \quad \mathbf{A}(\eta, \eta) \mathbf{G}_1(x)^2 + \mathbf{B}(\eta, \eta, \eta_1, \eta_2) \mathbf{G}_1(x) + \mathbf{C}(\eta, \eta) = 0.$$

Note that $\mathbf{A}(\eta, \eta)$, $\mathbf{B}(\eta, \eta, \eta_1, \eta_2)$ and $\mathbf{C}(\eta, \eta)$ are elements of the quadratic field extension $L/\mathbb{C}(x)$. Thus eq. (5.2) implies that $\mathbf{G}_1(x)$ is algebraic over L , that is, the field extension $L[\mathbf{G}_1(x)]/L$ is finite, whence the extension $L[\mathbf{G}_1(x)]/\mathbb{C}(x)$ is finite. Therefore $\mathbf{G}_1(x)$ is algebraic over $\mathbb{C}(x)$. Clearly this implies that $\mathbf{H}_\sigma(x)$ is algebraic over $\mathbb{C}(x)$ and in particular D -finite [20]. Pringsheim's Theorem [21] guarantees that $\mathbf{H}_\sigma(x)$ has a dominant real positive singularity κ_σ . We verify by explicit computation that for $1 \leq \sigma \leq 5$, the singularity κ_σ is the unique, minimal, positive real solution of the equation $\mathbf{Q}(x, \mathbf{T}_\sigma^{[\sigma+2]}(x)) = 0$ and a branch-point singularity of the square root. We list the values of κ_σ^{-1} in Table 1. Accordingly, at κ_σ , $\mathbf{H}_\sigma(x)$ coincides with its singular expansion and is given by

$$\mathbf{H}_\sigma(x) = h_0 + h_1(\kappa_\sigma - x)^{\frac{1}{2}} + O((\kappa_\sigma - x)).$$

Using Theorem 4 and Theorem 5, we arrive at

$$H_\sigma(s) \sim \frac{h_1(\kappa_\sigma)^{\frac{1}{2}}}{\Gamma(-\frac{1}{2})} s^{-\frac{3}{2}} (\kappa_\sigma)^{-s}.$$

Setting $c_\sigma = \frac{h_1(\kappa_\sigma)^{\frac{1}{2}}}{\Gamma(-\frac{1}{2})}$, we compute $c_1 \approx 1.38629$ and $c_2 \approx 3.51610$, completing the proof. \square

In Fig. 13, we showcase the quality of the asymptotic formula for $\sigma = 2$ and arc-length four, implied by Theorem 3.

6. DISCUSSION

In this paper we analyzed the biologically relevant class of canonical joint structures having arc-length greater than or equal to four. While it is straightforward to derive the generating function of joint structures from the (eleven) recursion relations of the original **rip**-grammar (implied by Proposition 1) [12] the generating function obtained this way would be “impossible” to write down. This approach would be neither suitable for deriving any asymptotic formulas nor would it allow us to deal with specific stack-length

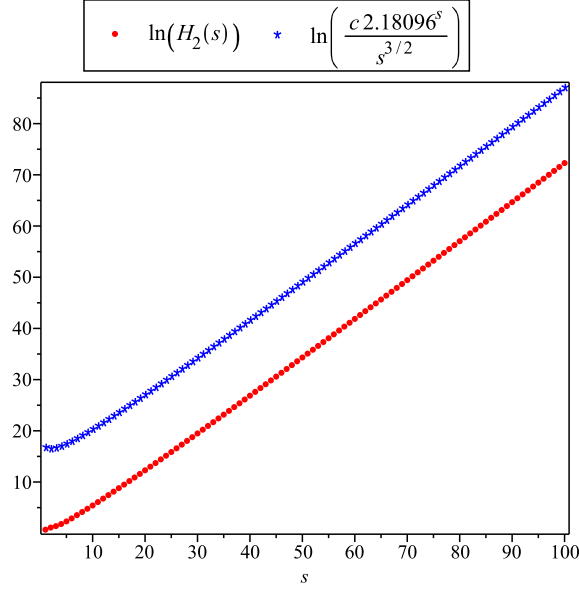


Fig. 13. Exact enumeration versus asymptotic formula. We contrast the numbers of 2-canonical joint structures with arc-length ≥ 4 ($H_2(s)$) versus $c s^{-\frac{3}{2}} 2.18096^s$. For representational purposes we separate the curves via setting the constant $c = 10^7$.

conditions. Therefore we do not use the recurrences implied by the rip-grammar [12]. Instead we build our theory as in [17] around the concept of shapes, which we “color”, in order to rule out certain (bad) inflation scenarios. Passing from shapes to refined shapes changes the shape-grammar as well as the underlying generating functions. The refined shapes are key to the generating functions since the collapsing of stems preserves vital information of the interaction structure. It is therefore not surprising that a shape induces joint structures via inflation, see Theorem 2.

As canonical joint structures of arc-length at least four constitute a novel combinatorial class it is of interest to compare them with the classes of RNA secondary structures (having generating function $\sum_n Q_2(s)z^s$) and 3-noncrossing pseudoknots structures ($\sum_n Q_3(s)z^s$). Here a 3-noncrossing structure has a diagram representation in which there are no three mutually crossing arcs. Indeed, clearly, RNA secondary structures are joint structures without any exterior arcs. Furthermore any joint structure can be interpreted as a particular 3-noncrossing structure, by rotating the bottom structure around its endpoint by 180 degrees, then aligning the two backbones and drawing all exterior arcs in the upper halfplane, see Fig. 14. For long sequences the numbers of canonical secondary structures, $Q_2(s)$ [22], joint structures $H_2(s)$ and 3-noncrossing pseudoknots structures $Q_3(s)$, all having arc-length at least four [18] we find

$$\begin{aligned} Q_2(s) &\sim 1.4848 s^{-\frac{3}{2}} 1.8489^s \\ H_2(s) &\sim 3.5161 s^{-\frac{3}{2}} 2.1801^s \\ Q_3(s) &\sim 5546 s^{-5} 2.5410^s, \end{aligned}$$

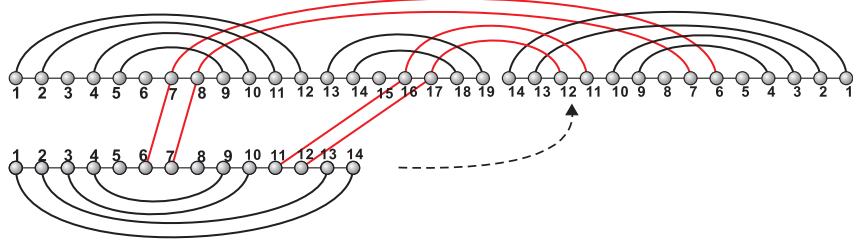


Fig. 14. Interpretation of joint structures as 3-noncrossing structures. A joint structure (left) can be represented as 3-noncrossing structure (top) by rotating the bottom sequence around its endpoint and aligning the two backbones and finally drawing all exterior arcs (red) in the upper halfplane.

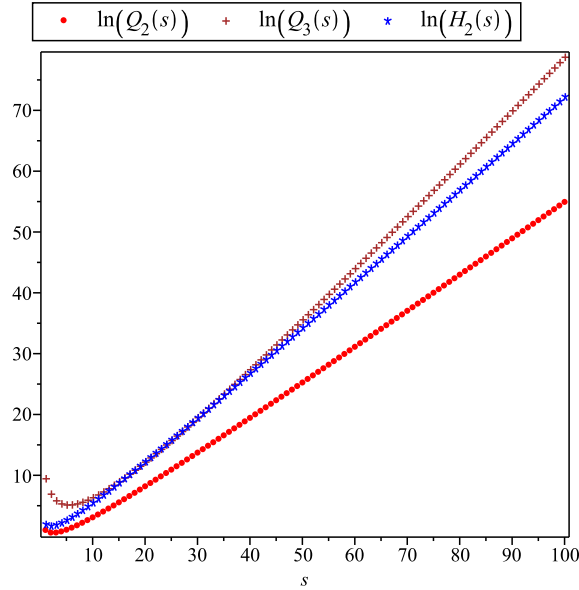


Fig. 15. How joint structures “fit” in: we display the numbers of secondary structures (red), joint structures (blue) and 3-noncrossing pseudoknots structures (brown). All structure classes are canonical and exhibit arc-length greater or equal to four.

see also Fig. 15.

We can report that joint structures resemble features of secondary structures as well as 3-noncrossing structures. Indeed, as it is the case for secondary structures, they can be MFE-folded in polynomial time and as RNA pseudoknot structures they exhibit crossing arcs and are truly shape-based structure class. However, in contrast to 3-noncrossing structures, refined shapes have algebraic generating functions (as opposed to D -finite ones) and satisfy simple recurrences.

Let us finally outline future research: with this paper the combinatorics of joint structures is completed. The next step is to study their topology, i.e. understanding how joint

structures filter via topological genera and boundary components. This program means to pass from the deformation retracts studied here to fat-graphs and their associated surfaces.

7. APPENDIX

7.1. Singularity analysis. In light of the fact that explicit formulas for the coefficients of a generating function can be very complicated or even impossible to obtain, we estimate of the coefficients in terms of the exponential factor and the subexponential factor. Singularity analysis gives a framework that allows to extract the asymptotics information of these coefficients. The key to obtain the asymptotic formulas about the coefficients of a generating function is its dominant singularities. The theorem of Pringsheim [23, 21] guarantees that a combinatorial generating function with nonnegative coefficients has its radius of convergence as its dominant singularity. Furthermore for all our generating functions it is the unique dominant singularity. The derivation of exponential growth rates and subexponential factors from singular expansions of generating functions mainly rely on the transfer theorems [23].

To be precise, we say a function $f(z)$ is Δ_ρ analytic at its dominant singularity $z = \rho$, if it analytic in some domain $\Delta_\rho(\phi, r) = \{z \mid |z| < r, z \neq \rho, |\text{Arg}(z - \rho)| > \phi\}$, for some ϕ, r , where $r > |\rho|$ and $0 < \phi < \frac{\pi}{2}$. We use the notation

$$(f(z) = \Theta(g(z)) \text{ as } z \rightarrow \rho) \iff (f(z)/g(z) \rightarrow c \text{ as } z \rightarrow \rho),$$

where c is some constant. Let $[z^n]f(z)$ denote the coefficient of z^n in the power series expansion of $f(z)$ at $z = 0$. Since the Taylor coefficients have the property

$$\forall \gamma \in \mathbb{C} \setminus 0; \quad [z^n]f(z) = \gamma^n [z^n]f\left(\frac{z}{\gamma}\right),$$

We can, without loss of generality, reduce our analysis to the case where $z = 1$ is the unique dominant singularity. The following theorems transfer the asymptotic expansion of a function around its unique dominant singularity to the asymptotic of the function's coefficients.

Theorem 4. [23] *Let $f(z)$ be a Δ_1 analytic function at its unique dominant singularity $z = 1$. Let*

$$g(z) = (1 - z)^\alpha \log^\beta \left(\frac{1}{1 - z} \right), \quad \alpha, \beta \in \mathbb{R}.$$

That is we have in the intersection of a neighborhood of 1

$$(7.1) \quad f(z) = \Theta(g(z)) \quad \text{for } z \rightarrow 1.$$

Then we have

$$(7.2) \quad [z^n]f(z) = \Theta([z^n]g(z)).$$

Theorem 5. [23] Suppose $f(z) = (1 - z)^{-\alpha}$, $\alpha \in \mathbb{C} \setminus \mathbb{Z}_{\leq 0}$, then

$$(7.3) \quad f(z) \sim \frac{n^{\alpha-1}}{\Gamma(\alpha)} \left[1 + \frac{\alpha(\alpha-1)}{2n} + \frac{\alpha(\alpha-1)(\alpha-2)(3\alpha-1)}{24n^2} + \frac{\alpha^2(\alpha-1)^2(\alpha-2)(\alpha-3)}{48n^3} + O\left(\frac{1}{n^4}\right) \right].$$

7.2. Symbolic Enumeration. Symbolic enumeration [23] plays an important role in the computations of generating functions. We first introduce the notion of a combinatorial class. Let $\mathbf{z} = (z_1, \dots, z_d)$ be a vector of d formal variables and $\mathbf{k} = (k_1, \dots, k_d)$ be a vector of integers of the same dimension. We use the simplified notation

$$\mathbf{z}^{\mathbf{k}} := z_1^{k_1} \cdots z_d^{k_d}.$$

Definition 1. A combinatorial class of d dimension, or simply a class, is an ordered pair $(\mathcal{A}, w_{\mathcal{A}})$ where \mathcal{A} is a finite or denumerable set and a size-function $w_{\mathcal{A}}: \mathcal{A} \rightarrow \mathbb{Z}_{\geq 0}^d$ satisfies that $w_{\mathcal{A}}^{-1}(\mathbf{n})$ is finite for any $\mathbf{n} \in \mathbb{Z}_{\geq 0}^d$.

Given a class $(\mathcal{A}, w_{\mathcal{A}})$, the size of an element $a \in \mathcal{A}$ is denoted by $w_{\mathcal{A}}(a)$, or simply $w(a)$. We consistently denote by $\mathcal{A}_{\mathbf{n}}$ the set of elements in \mathcal{A} that have size \mathbf{n} and use the same group of letters for the cardinality $A_{\mathbf{n}} = |\mathcal{A}_{\mathbf{n}}|$. The sequence $\{A_{\mathbf{n}}\}$ is called the counting sequence of class \mathcal{A} . The generating function of a class $(\mathcal{A}, w_{\mathcal{A}})$ is given by

$$\mathbf{A}(\mathbf{z}) = \sum_{a \in \mathcal{A}} \mathbf{z}^{w_{\mathcal{A}}(a)} = \sum_{\mathbf{n}} A_{\mathbf{n}} \mathbf{z}^{\mathbf{n}}.$$

There are two special classes: \mathcal{E} and \mathcal{Z}_i which contain only one element of size $\mathbf{0}$ and \mathbf{e}_i , respectively. In particular, the generating functions of the classes \mathcal{E} and \mathcal{Z}_i are

$$\mathbf{E}(\mathbf{z}) = 1 \quad \text{and} \quad \mathbf{Z}_i(\mathbf{z}) = z_i.$$

Next we introduce some basic constructions that constitute the core of a specification language for combinatorial structures. Let \mathcal{A} and \mathcal{B} be combinatorial classes of d dimension. Suppose \mathcal{A}_i are combinatorial classes of 1 dimension. We define

- $(\mathcal{A}_1, \mathcal{A}_2) := \{c = (a_1, a_2) \mid a_i \in \mathcal{A}_i\}$ and for $c = (a_1, a_2) \in (\mathcal{A}_1, \mathcal{A}_2)$

$$w_{(\mathcal{A}_1, \mathcal{A}_2)}(c) = (w_{\mathcal{A}_1}(a_1), w_{\mathcal{A}_2}(a_2)),$$

- $\mathcal{A} + \mathcal{B} := \mathcal{A} \cup \mathcal{B}$, if $\mathcal{A} \cap \mathcal{B} = \emptyset$ and for $c \in \mathcal{A} + \mathcal{B}$,

$$w_{\mathcal{A}+\mathcal{B}}(c) = \begin{cases} w_{\mathcal{A}}(c) & \text{if } c \in \mathcal{A} \\ w_{\mathcal{B}}(c) & \text{if } c \in \mathcal{B}, \end{cases}$$

- $\mathcal{A} \times \mathcal{B} := \{c = (a, b) \mid a \in \mathcal{A}, b \in \mathcal{B}\}$ and for $c \in \mathcal{A} \times \mathcal{B}$,

$$w_{\mathcal{A} \times \mathcal{B}}(c) = w_{\mathcal{A}}(a) + w_{\mathcal{B}}(b),$$

- $\text{SEQ}(\mathcal{A}) := \mathcal{E} + \mathcal{A} + (\mathcal{A} \times \mathcal{A}) + (\mathcal{A} \times \mathcal{A} \times \mathcal{A}) + \cdots$.

Plainly, $\text{SEQ}(\mathcal{A})$ defines a proper combinatorial class if and only if \mathcal{A} contains no element of size 0. We immediately observe

Proposition 2. Suppose \mathcal{A} , \mathcal{B} and \mathcal{C} are combinatorial classes of d dimension having the generating functions $\mathbf{A}(\mathbf{z})$, $\mathbf{B}(\mathbf{z})$ and $\mathbf{C}(\mathbf{z})$. Let \mathcal{A}_i be combinatorial classes of 1 dimension having the generating functions $\mathbf{A}_i(z)$. Then

- (a) $\mathcal{C} = (\mathcal{A}_1, \mathcal{A}_2, \dots, \mathcal{A}_d) \implies \mathbf{C}(\mathbf{z}) = \mathbf{A}_1(z_1) \mathbf{A}_2(z_2) \dots \mathbf{A}_d(z_d)$
- (b) $\mathcal{C} = \mathcal{A} + \mathcal{B} \implies \mathbf{C}(\mathbf{z}) = \mathbf{A}(\mathbf{z}) + \mathbf{B}(\mathbf{z})$
- (c) $\mathcal{C} = \mathcal{A} \times \mathcal{B} \implies \mathbf{C}(\mathbf{z}) = \mathbf{A}(\mathbf{z}) \cdot \mathbf{B}(\mathbf{z})$
- (d) $\mathcal{C} = \text{SEQ}(\mathcal{A}) \implies \mathbf{C}(\mathbf{z}) = \frac{1}{1-\mathbf{A}(\mathbf{z})}$.

REFERENCES

- [1] F. Narberhaus, J. Vogel, Sensory and regulatory RNAs in prokaryotes: A new german research focus, RNA Biol. 4 (2007) 160–164.
- [2] M.T. McManus, P.A. Sharp, Gene silencing in mammals by small interfering RNAs, Nature Reviews 3 (2002) 737–747.
- [3] D. Banerjee, F. Slack, Control of developmental timing by small temporal RNAs: a paradigm for RNA-mediated regulation of gene expression, Bioessays 24 (2002) 119–129.
- [4] J.P. Bachellerie, J. Cavaillé, A. Hüttenhofer, The expanding snoRNA world, Biochimie 84 (2002) 775–790.
- [5] R. Benne, RNA editing in trypanosomes. the use of guide RNAs, Mol. Biol. Rep. 16 (1992) 217–227.
- [6] J.F. Kugel, J.A. Goodrich, An RNA transcriptional regulator templates its own regulatory RNA, Nat. Struct. Mol. Biol. 3 (2007) 89–90.
- [7] B. Hekimoglu, L. Ringrose, Non-coding RNAs in polycomb/trithorax regulation, RNA Biol. 6 (2009) 129–137.
- [8] C. Alkan, E. Karakoc, J.H. Nadeau, S.C. Sahinalp, K. Zhang, RNA-RNA interaction prediction and antisense RNA target search, J. Comput. Biol. 13 (2006) 267–282.
- [9] D.D. Pervouchine, IRIS: Intermolecular RNA interaction search, Proc. Genome Informatics 15 (2004) 92–101.
- [10] I. Lebars, P. Legrand, A. Aimé, N. Pinaud, S. Fribourg, C. Di Primo, Exploring TAR-RNA aptamer loop-loop interaction by X-ray crystallography, UV spectroscopy and surface plasmon resonance, Nucleic Acids Res. 36 (2008) 7146–7156.
- [11] R. Salari, R. Backofen, S.C. Sahinalp, Fast prediction of RNA-RNA interaction, Algorithms Mol Biol (2010) doi:10.1186/1748-7188-5-5.
- [12] F.W.D. Huang, J. Qin, P.F. Stadler, C.M. Reidys, Partition function and base pairing probabilities for RNA-RNA interaction prediction, Bioinformatics 25 (2009) 2646–2654.
- [13] H. Chitsaz, R. Salari, S.C. Sahinalp, R. Backofen, A partition function algorithm for interacting nucleic acid strands, Bioinformatics 25 (2009) i365–i373.
- [14] F.W.D. Huang, J. Qin, P.F. Stadler, C.M. Reidys, Target prediction and a statistical sampling algorithm for RNA-RNA interaction, Bioinformatics 26 (2010) 175–181.
- [15] M.S. Waterman, T.F. Smith, RNA secondary structure: A complete mathematical analysis, Math. Biosci 42 (1978) 257–266.
- [16] W.R. Schmitt, M.S. Waterman, Linear trees and RNA secondary structure, Disc. Appl. Math. 51 (1994) 317–323.
- [17] T.J.X. Li, C.M. Reidys, Combinatorics of RNA-RNA interaction, in press arXiv:1006.2924v1.
- [18] C.M. Reidys, R.R. Wang, A.Y.Y. Zhao, Modular, k -noncrossing diagrams, Electron. J. Combin. (2010).
- [19] E.Y. Jin, C.M. Reidys, Combinatorial design of pseudoknot RNA, Adv. Appl. Math. 42 (2009) 135–151.
- [20] R.P. Stanley, Enumerative Combinatorics, Volume 2, Cambridge University Press, Cambridge, England, 1999.
- [21] E.C. Titchmarsh, The theory of functions, Oxford University Press, Oxford UK, 1939.

- [22] I.L. Hofacker, P. Schuster, P.F. Stadler Combinatorics of RNA secondary structures, *Discr. Appl. Math.* 88 (1998) 207–237.
- [23] P. Flajolet, R. Sedgewick, *Analytic combinatorics*, Cambridge University Press, 2008.